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SOME STRUCTURAL TRANSFORMATIONS OF THE BLOOD-CELLS OF VERTEBRATES *

G. L. KITE

(From the Henry Phipps Institute of the University of Pennsylvania, Philadelphia, Pa.)

PLATE 2

The current descriptions of the structure of vertebrate blood-cells are largely based on material which has been prepared by the smear method and coagulated by heat, alcohol, or mixtures of electrolytes. The activities of white cells, that are generally recognized, are slow ameboid movement by means of either blunt or fine pseudopods, and the engulfment of objects by means of active pseudopods.

The numerous recent investigations on blood-cells made with the aid of the dark-field method of illumination have added interesting points of detail to previous knowledge of the form and activities of the cellular elements of the blood. No convincing evidence has been brought forward to show that the widely held notions about vertebrate blood-cells are seriously at fault. This fact is clearly due to the confusion caused by the presence of fibrin granules and threads in preparations made by the usual methods. In fact, many of the recent investigations are nothing more than the description of attempts to interpret the complex and variable images of the structural elements of blood clots which are produced by oblique illumination on a dark background.

By the employment of a special mounting or culture medium, I have been enabled to determine that both white cells and erythrocytes of all classes of vertebrates may undergo many marked structural transformations, which are of a reversible nature. In this paper, the more important of these transformations, so far observed, will be described.

MATERIAL AND METHODS

The blood-cells of fishes, amphibians, reptiles, birds, and mammals have been studied. Particular attention has been given to the blood-cells of the frog, the rabbit, and man. The actual species, so far used, include common mammals, as the mouse, goat, guinea-pig, rabbit, monkey (*Macacus rhesus*), and man, and in addition the goldfish

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(*Carassius auratus*), bullfrog tadpole (*Rana catesbiana*), leopard frog (*Rana pipiens*), painted turtle (*Chrysemis picta*), and pigeon (*Columba livia*).

A detailed description of the microdissection method employed will soon be published. It will suffice to state that the method has been developed to the point where it is adequate for the determination of the colloidal structure of living matter. Such structural elements of living cells as nuclei, nucleoli, pieces of chromatin networks, globules, granules, chromosomes, and spindle fibers may be cut out of living animal and plant cells and their exact physical properties and relationships determined. A new microdissection apparatus has been designed embodying the well-known ball-bearing principle. The three micrometer screws in this apparatus are made with the accuracy common to the best microtomes. Special achromatic and apochromatic condensers, giving long working distances, are essential for the dissection of cells under oil immersion objectives.

A 2 mm. Zeiss objective of 1.40 N. A., Comp. Ocs. 6 and 8, and W. Watson and Sons' holoscopic oil-immersion condenser, have been used for observing living blood-cells and for microdissections. The dark-field method of illumination has also been employed with notable success.

Liquid plasma, diluted with Ringer's fluid, which contains sufficient hirudin to permanently prevent coagulation, has been generally employed as the mounting medium. The technic which I have found best for the preparation of the blood-cells for observation and microdissection is very simple. About one milligram of hirudin is added to 2 or 3 c.c. of sterile Ringer's fluid in a small flask. About 0.5 c.c. of this mixture is drawn into a pipet which is applied to a drop of fresh blood obtained by the usual puncture method. The blood and Ringer's fluid are quickly blown into the flask containing the Ringer's fluid and hirudin. This operation is repeated until the Ringer's fluid has a definite red color. If sufficient hirudin has been added to the Ringer's fluid, and reasonable deftness be shown in getting the blood well mixed with the Ringer's fluid, neither fibrin granules nor threads are formed and the platelets are not clumped. Microscopical examination of a drop of this mixture should show the blood-cells evenly distributed through the liquid, and widely enough spaced to give optimum conditions for the exhibition of activities by the cells.

This use of hirudin is based on the work of Bodong,¹ and Abel, Rowntree, and Turner.² Bodong has demonstrated the harmlessness of even large quantities of hirudin for rabbits, and Abel, Rowntree, and Turner could show no detrimental action of this substance on dogs. Hirudin has given equally satisfactory results with the blood-cells of all vertebrates so far tried. The simple method of using hirudin that I have described is so satisfactory that it may find wide usage among those interested in the microscopic examination of fresh blood. Further, it seems that plasma, kept liquid by the employment of hirudin, used either undiluted or diluted with an appropriate salt solution may prove of great value in the *in vitro* cultivation of many other types of cells, besides the formed elements of the blood.

The results obtained by the hirudin method have been controlled by the use of defibrinated blood and by the addition of citrate and oxalate to prevent coagulation. While similar physical changes of the blood-cells may be observed when these more usual methods are employed, no evidence could be adduced to show that such methods of handling blood-cells for *in-vitro* studies have anything but disadvantages as compared with liquid-plasma and Ringer's fluid containing a trace of hirudin.

The observations and microdissections of the blood-cells of cold-blooded vertebrates were made at room temperature; those of birds and mammals at both room temperature and at body temperature. The structural transformations of blood-cells are so similar for all classes of vertebrates that the descriptions may be largely limited to those of the rabbit and man.

THE COLLOIDAL STRUCTURE OF THE CYTOPLASM OF HUMAN POLYMORPHONUCLEAR LEUKOCYTES

Microdissections of polymorphonuclear leukocytes, under oil-immersion objectives, have shown that the usual descriptions of their structure are entirely erroneous. The cytoplasm of the white cells of all classes of vertebrates has nothing of the nature of a cell membrane. The surfaces of these cells are as completely naked as those of Rhizopods, nor does the living leukocyte cytoplasm contain a spongioplasm and hyaloplasm. The cytoplasm of this cell is a jelly in which are imbedded large numbers of globules. A very thin sur-

1. *Arch. f. exper. Path. u. Pharmacol.*, 1904, 52, p. 242.
2. *Jour. pharmacol. and Exp. Therapy*, 1913, 5, p. 275.

face layer of cytoplasm goes more or less completely into solution when the surface is incised or extensively torn with glass dissecting needles. This change is so rapid and affects such a small mass of the living cytoplasmic jelly that it may be easily overlooked even when the dissections are made under oil-immersion objectives. With this exception the cytoplasm of polymorphonuclear leukocytes does not change rapidly as a result of mechanical injury. If pieces of the living cytoplasmic jelly between the globules are torn out, they are found to be optically homogeneous or monophasic in molar structure. The well-known turbidity of the living polymorphonuclear leukocyte is due to the globular component; the cytoplasm proper is almost transparent and has extremely little scattering action on light. The structures usually termed cytoplasmic granules are of the nature of separation products; they do not grade into the surrounding cytoplasm. In my terminology, such components of the living substance are termed globules or spherules while those that grade into the imbedding substance are termed granules. This is believed to be a fundamental physical distinction. It is almost impossible to free the globules entirely from the remarkably glutinous cytoplasm. The substances that form the globules change their distribution in the different phases of the leukocyte. In one phase, the globules are localized in one small area of cytoplasm. In other phases, the globules vary greatly in size in different parts of the same cell. The structural death-changes are also commonly associated with marked redistribution of the globular material.³ Spermatzoa and the spermatocytes of insects are the only cells, so far dissected, that may be compared in glutinosity to leukocytes. The cytoplasm is soft and may be easily pulled out into long strands which contract considerably when freed. Pieces of the cytoplasmic jelly do not round up when torn out in diluted plasma. Jams made from colorless berries show physical and optical properties that are in many ways similar to those of the cytoplasm of leukocytes.

STRUCTURAL TRANSFORMATIONS OF POLYMORPHONUCLEAR LEUKOCYTES

The citation of an actual experiment may serve to illustrate the nature of the structural transformations observed. A drop of diluted human blood prepared according to the hirudin method was mounted and sealed with vaselin. The preparation was immediately trans-

3. For similar observations see Schilling, *Folia haematol.*, 1908, 6, p. 429.

ferred to a microscope incubator and examined by dark field illumination. Most of the leukocytes extend blunt pseudopods and by this means flow slowly across the field. Inside of fifteen minutes many leukocytes may be observed to undergo a marked surface change. A surface layer of from one to three or more microns in thickness becomes

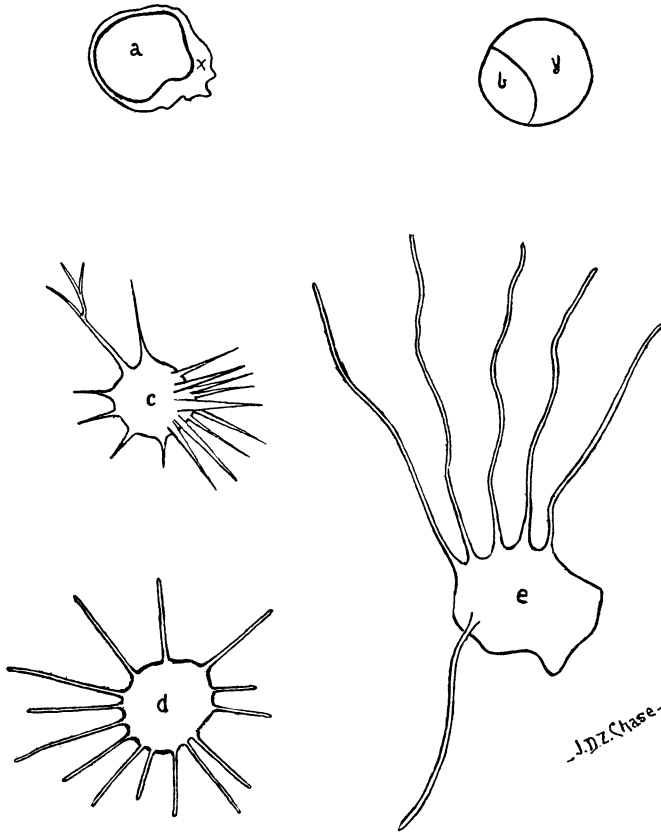


Fig. 1.—Camera drawings of living human polymorphonuclear leukocytes.

a=Hyaline-surface phase; x=Hyaline surface layer; b=Hyaline phase; y=Hyaline area; c=Fine pseudopodial phase; d=Ciliated phase; e=Flagellated phase. The processes usually have an irregular distribution over the surface of the leukocytes, but this important structural detail could not be brought out well in the camera drawings.

All camera drawings were made with 1.8 mm. Zeiss homogeneous immersion objective, No. 6 compensating ocular, and paraboloid condenser.

optically delineated from the underlying substance. This stage is shown in Figure 1, *a*. In less than thirty minutes many leukocytes are seen, covered with delicate protoplasmic processes which may assume

at least three different forms, namely, fine, long immobile pseudopods that frequently branch, short cilium-like processes, and long delicate processes which have the rapid undulatory movement characteristic of flagella. The protrusion and retraction of the different kinds of processes have been observed many times. Leukocytes with as many as twelve or fifteen long undulating processes in one focal plane have been observed to retract all processes in the course of a few seconds and become typical ameboid cells.

The more important distinct forms or phases of the leukocyte are shown diagrammatically in Figures 1 and 2. The hyaline surface phase is characterized by an extraordinary viscosity of the surface. If two leukocytes in this phase are brought in contact, fusion at the

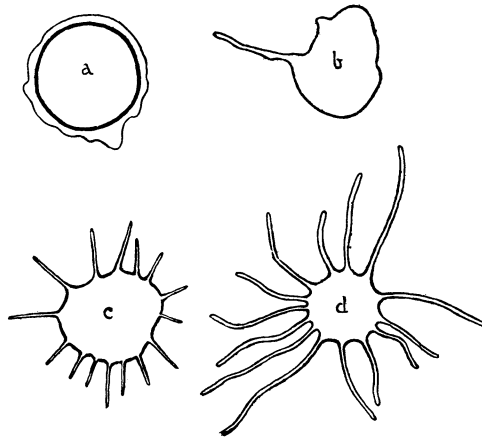


Fig. 2.—Camera drawings of living polymorphonuclear leukocytes of the rabbit.
a = Hyaline-surface phase; b and c = Ciliated phase; d = Flagellated phase.

point of contact occurs. If the surface of such a leukocyte is touched with a glass dissecting needle and the needle drawn away, a minute thread of hyaline cytoplasm may be seen connecting the point of the needle with the leukocyte. When motile bacteria strike this layer they stick and generally do not seem to be able to free themselves. The violent jerking movements made by the bacteria may serve to imbed them firmly in the surface of the leukocytes. Observations of the mesenteries of frogs, exposed to air, indicate that the well-known accumulation of leukocytes on the walls of the small blood-vessels is due, primarily, to a marked increase in viscosity of the surface layers

of the leukocyte cytoplasm. The important power of undergoing rapid variation in surface viscosity, in response to slight environmental changes, is not limited to the white blood-cells of vertebrates, since Hardy⁴ has given an excellent description of this phenomenon in the blood-cells of *Daphnia*.

The pseudopodial phase of a human polymorphonuclear leukocyte is shown in Figure 1. Processes of this type are subject to the widest variation and in some cases reach extraordinary lengths. In 1891 Ranvier⁵ described frog lymphocytes with numerous long pseudopods, under the name of "clasmatocytes." Ranvier considers clasmatocytes to be new types of cells. It appears that retraction of the pseudopods was not observed. More recently Joly⁶ has figured the clasmatocytes of Ranvier.

In the ciliated phase the processes are usually numerous and vary in length from about four to ten microns. The processes exhibit an uncoordinated cilium-like beating. A part of the processes on a cell may be motionless, while others show rapid movement. When the processes reach a length of from ten to thirty or more microns they have the undulatory motion common to flagella. Such forms may be considered flagellated phases. Both the ciliated and flagellated phases may be free-swimming. On account of the lack of coordination in the movements of the processes, such leukocytes follow a very irregular path when moving across the field.

As far as I have been able to determine, both mononuclear and polymorphonuclear white blood-cells of all classes of vertebrates may undergo these strange changes. A peculiar redistribution change that has been frequently observed is characterized by the hyaline character of the greater part of the cell-substance. In this phase the globules are localized in a small area of the cytoplasm.

In this connection two recent papers that have a general bearing on structural transformations in cells may be mentioned. Holmes⁷ has described the transformation of hexagonal peritoneal epithelial cells of the frog into ciliated cells which in turn become ameboid. Holmes writes, "One would not suspect these (ameboid) cells to be derived from ciliated epithelium were it not for the fact that one can actually observe their transformation." In an important study of an ameba

4. *Jour. Physiol.*, 1892, 13, p. 165.

5. *Compt. rend. Acad. d. sc.*, 1891, 112, p. 688.

6. *Arch de méd. expér. et d'anat. path.*, 1898, 10, p. 616.

7. *Science, New Series*, 1914, 39, p. 107.

of the limax group, Wherry⁸ describes the transformation under certain conditions of typical amebae into flagellates. Wherry states that the pressure of a cover glass was generally sufficient to cause the change of the free-swimming flagellated forms back into amebae.

STRUCTURAL CHANGES IN MYELOCYTES

A number of observations have been made on the blood-cells from a case of myelogenous leukemia. Myelocytes with pseudopods and long flagellum-like processes are shown in Figures 1 and 3, Plate 2. In preparations of leukemic blood, prepared by the hirudin method, the myelocytes seemed to be the first cells to extrude processes. Large myelocytes, covered with numerous long undulating processes, make striking microscopic pictures.

In both normal and leukemic blood a small body, about three microns in diameter, containing a few granules and having from one to four long delicate processes, has been observed. The exact identity of this structure has not been determined.

THE BEHAVIOR OF UNATTACHED PROCESSES

Processes that have lost their connection with cells have an independent undulatory movement. A single free process has been observed to retain its characteristic activity unimpaired for over one hour. A process that lies obliquely in the focal plane of an objective actually simulates a spirochete. Such unattached processes seem undoubtedly to be a source of the pseudo-spirochetes of normal blood, so familiar to those investigators who work much with dark field illumination.

It appears certain that active unattached processes have been observed by Rosenthal,⁹ Beer,¹⁰ Chambers,¹¹ Ketron,¹² and others; yet, it should be recalled that preparations made according to the usual methods contain plain and beaded threads of fibrin, which may closely simulate the unattached processes of blood-cells. Besides, Ketron states, "If blood is allowed to flow in a citrate solution to prevent clotting none of these bodies are found and I was not able to produce them by trauma." And further, "These results seem to give definite proof that these pseudo-organisms are most likely products of fibrin formation."

8. *Arch. f. Protistenkunde*, 1913, 31, p. 77.

9. J. Rosenthal, *Festschrift*, Leipzig, 1906.

10. *München. med. Wchnschr.*, 1907, 54, p. 1926.

11. *Lancet*, 1913, 1, p. 1728.

12. *Jour. Cutan. Dis.*, 1914, 32, p. 216.

It may be relevant to note that observers should be able to distinguish between brownian movement about a mean position and the motion exhibited by active living cell processes—either attached or free.

STRUCTURAL CHANGES IN ERYTHROCYTES

A photomicrograph of a living human erythrocyte with several processes is shown in Figure 2, Plate 2. The long process showed typical flagellum-like movement just before the exposure for the photomicrograph was made. The short processes on the same erythrocyte were not well brought out on account of their rapid movement. A red cell with such processes may be actually slowly free-swimming. The processes observed on the erythrocytes of birds, turtles, frogs, and fishes were particularly long and beautiful. As many as fifteen short processes have been seen in one focal plane on a human erythrocyte. Nucleated red cells usually have from one to four very clear or hyaline processes. The protrusion and retraction of the processes have been observed for both nucleated and non-nucleated red corpuscles. Occasionally a long process is completely retracted in much less than a second.

Certain peculiar redistribution phenomena, which occur in sealed preparations of red corpuscles of all classes of vertebrates, are worthy of a brief description. Such structures as granules, rings, curved rods, and undulating filaments, have been seen in the red cells of all vertebrates below mammals. Even the nuclear substance may change its distribution. Changes of the same order, but much less marked, are common in sealed preparations of human red cells. Granules frequently separate out of the corpuscular jelly, and notable changes occur in the distribution and density of the surface layers.

THE CONDITIONS UNDER WHICH VERTEBRATE BLOOD-CELLS UNDERGO STRUCTURAL TRANSFORMATIONS

The conditions under which cell-processes are put out have proved very variable. Human and rabbit white blood-cells, which have remained at room temperature from one and one-half to two hours after removal from the body, usually show all the phases that I have described, when examined in sealed preparations by dark field illumination, at either incubator or room temperature. The change from body to room temperatures seems to have a peculiar effect on the

white cells of birds and mammals. For the first one or two hours after removal from the body, these cells are usually either inactive or show only ameboid movement and the hyaline surface phase. Incubator temperature may greatly shorten the time of appearance of processes. White blood-cells with processes have been observed in pieces of living tubercles from mouse lung; in exudates from corneal tubercles; in the mesenteries of frogs, which had been exposed to the air for several hours; and in samples of pus.

Very little has been made out about the precise conditions under which mammalian red corpuscles and nucleated erythrocytes of the other classes of vertebrates extrude processes. Human red corpuscles at room temperature do not usually show processes for some hours. In one preparation of human blood, kept at room temperature for about four hours, almost every red corpuscle had a single long undulating process. In the course of a thirty-minute observation many of the processes were retracted. The nucleated red cells of pigeons, turtles, frogs, and fishes, when mounted and sealed, and kept at room temperature, showed processes in from three hours to two days or even longer. Many human and nucleated red cells with undulating processes, which had lost their hemaglobin and were almost invisible, were observed. Certainly both nucleated and non-nucleated red corpuscles may be very active after all visible hemaglobin is lost. A decrease in oxygen tension appears to be a factor of some importance in the production of transformations in white and red blood-cells. I have recently succeeded in demonstrating the presence of numerous processes on white blood-cells which were mounted in shallow drops in a moist chamber and left for one hour at room temperature and then subjected to a temperature of 0 C. and fixed at this temperature with osmic vapor.

THE EFFECT OF LOW OXYGEN PRESSURE ON THE ACTIVITY OF BLOOD-CELLS

An extensive usage of sealed preparations has brought out the important fact that the white and red cells of vertebrates have the power of adjusting their metabolism to very low partial pressures of oxygen and even to anaerobic conditions. Very active red and white cells have been observed in sealed preparations kept at room temperature for three days.

THE RELATION OF STRUCTURAL CHANGES IN LEUKOCYTES
TO PHAGOCYTOSIS

A number of experiments have been made with the aim of gaining if possible a clearer insight into the mechanism of the phagocytosis of bacteria by leukocytes and of determining the exact form taken by leukocytes in conditions similar to those of the usual in-vitro methods of studying phagocytosis. The results are of considerable interest. Leukocytes prepared according to the opsonic technic, mounted and sealed, and examined on a dark field in a microscope incubator, usually show the hyaline surface change in from ten to twenty minutes. White blood-cells in this phase frequently exhibit a rapid oscillatory flowing of parts of the surface jelly. The movement of globules in this layer has been frequently misinterpreted by previous observers and considered brownian movement. Leukocytes with numerous moving processes have been observed in such preparations in less than ten minutes after their removal to the incubator. The addition of emulsions of staphylococci to leukocytes does not change their behavior in any way that I could determine. In preparations of mixtures of motile bacteria and white blood-cells containing ciliated or flagellated forms, the actual entanglement of the bacteria by the processes was observed a great number of times. Occasionally the violent struggle of a bacterium resulted in freeing it from the leukocyte instead of more thoroughly imbedding it in the surfaces of the processes, as usually seemed to be the case. Further, the results of all my experiments on in-vitro phagocytosis point to the varying viscosity of the surface layers of the white cells as a fundamental factor in the fixing of bacteria to their surfaces. The oscillatory flowing of parts of the cytoplasm may well prove to be capable of carrying bacteria into the deeper layers of the leukocyte.

Finally it seems well to emphasize some of the optical difficulties which have been met in the course of this investigation. It is a well-known principle of physical optics that transparent bodies, which have the same refractive index and dispersive power as the mounting medium, are invisible when viewed by transmitted light. This important optical principle holds for many of the structural changes in blood-cells that I have described. Redistribution of the globular substance is about all that can be seen with achromatic objectives and uncorrected condensers. With the notable exception of cilium- and

flagellum-like processes, about all the structural transformations may be observed by transmitted light with the optical system described above.

All the structural changes in blood-cells that I have figured or described may be observed with dark field illumination, yet this method has only a qualitative value. Processes on the upper and lower surfaces of cells are usually invisible. The long hyaline processes are likewise invisible unless the background is intensely black and they lie well within the focal plane. Another serious limitation of dark field illumination is the frequent failure of this optical method to bring out the exact structural relations of rapidly moving processes to the cell body. This particular limitation seems to be general, since an examination of numerous ciliates and flagellates gave similar results.

I am indebted to Dr. Paul A. Lewis for helpful criticisms and to Prof. C. E. McClung for the photographs.

EXPLANATION OF PLATE 2

Fig. 1.—Photomicrograph of blood-cells from a case of myelogenous leukemia.

Blood-cells prepared by the hirudin method, and fixed wet, at about 0° C. with osmic vapor and stained with iron hematoxylin. Myelocyte showing three long delicate processes. Magnified about 1000 diameters.

Fig. 2.—Photomicrograph of living human erythrocytes on dark field.

An erythrocyte with one long and several short processes. Magnified about 1000 diameters.

Fig. 3.—Drawing of myelocytes.

a=A large myelocyte covered with fine pseudopods; b=A medium-sized myelocyte with long delicate processes of the flagellum type. The granules on two of the processes are probably artefacts, as such structures were not observed in living, active myelocytes. The leukemic blood was prepared by the hirudin method and fixed wet, at low temperature, with osmic vapor and stained with iron hematoxylin.

Magnified about 750 diameters.